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(57) Abstract

DNA sequences which encode the enzyme solanidine UDP–glucose glucosyltransferase (SGT) are disclosed. Recombinant DNA molecules containing the sequences, and use thereof, in particular, use of an antisense DNA construct to inhibit the production of SGT and thereby reduce glycoalkaloid levels in solanaceous plants, e.g., potato, are described.

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DNA SEQUENCES ENCODING SOLANIDINE UDP-GLUCOSE GLUCOSYLTRANSFERASE AND USE TO REDUCE GLYCOALKALOIDS IN SOLANACEOUS PLANTS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the enzyme solanidine UDP-glucose glucosyltransferase (SGT) which is involved in the biosynthesis of steroidal glycoalkaloids in solanaceous plants. More particularly, the invention relates to DNA sequences which encode SGT, recombinant polynucleotide molecules containing the sequences, and use thereof, in particular, use of an antisense DNA construct to inhibit SGT activity and glycoalkaloid levels in solanaceous plants.

Description of the Art

Solanaceous plants include such agronomically important crops as potato and tomato. Solanaceous species synthesize steroidal glycoalkaloids (nitrogen-containing steroidal glycosides) which are natural toxicants and are believed to serve as natural defenses against insect and other pests. These compounds can exhibit toxic effects in humans as well as insects, and thus represent a potential source of toxicants, especially in improperly stored or processed potatoes. This has led to the implementation of a guideline limiting glycoalkaloid content in a tuber of a given potato cultivar to 20 mg/100 gm. Worldwide, between 13 and 27% of the potato crop has to be discarded because glycoalkaloid levels are above the maximum currently deemed to be safe (Morris and Lee, Food Technol. Aust. 36: 118-124 (1984)). While the guideline provides effective protection for the consumer, its effectiveness is dependent upon limiting the release of new cultivars for commercial production to those with

acceptable glycoalkaloid levels. For potato breeding programs to develop new cultivars with improved agronomic or processing properties, the need to select also for low levels of glycoalkaloids can present a difficult problem. A method to decrease the glycoalkaloid content of any newly developed cultivar with minimum impact on other characteristics would be of great use to obtain valuable new commercial potato cultivars.

In cultivated potato the predominant glycoalkaloid species, α-chaconine and α-solanine, are triglycosylated derivatives of the aglycon solanidine. These steroidal glycoalkaloids (SGAs) contain either glucose (α-chaconine) or galactose (α-solanine) as the primary glycosyl residue. A simplified biochemical pathway illustrating biosynthesis of the toxic glycoalkaloids α-chaconine and α-solanine is shown in FIG. 1. The synthesis of γ-chaconine (3-ß-O-glucosylsolanidine), an intermediate in the pathway to α-chaconine, is catalyzed by SGT. The activity and kinetics of the enzyme SGT have been characterized by a number of groups (Stapleton *et al.*, J. Agric. Food Chem. 39:1187-1193 (1991); Bergenstråhle *et al.*, Plant Sci. 84:35-44 (1992); Zimowski, Phytochemistry 6:1827-1831 (1991); Packowski and Wojciechowski, Phytochemistry 35:1429-1434 (1994)). The aglycone portion of the glycoalkaloid is believed to be considerably less toxic than the glycoside. It is believed that decreasing the activity of the enzyme(s) responsible for glycosylation of the aglycone should effectively lower the potential toxicity of potato cultivars.

A biosynthetic pathway to solanidine has been proposed (Kaneko *et al.*, Phytochemistry 15: 1391-1393 (1976), E. Heftmann, Phytochemistry 22: 1843-1860 (1983)). Information on the enzymatic mechanisms involved in the glycosylation steps of solanidine to form glycoalkaloids is limited to reports utilizing

relatively crude enzyme preparations (D. R. Liljegren, Phytochemistry 10: 3061-3064 (1971), Jadhav *et al.*, Journal of Food Science 38: 1099 (1973), Lavintman *et al.*, Plant Science Letters 8: 65-70 (1977), Osman *et al.*, Phytochemistry 19: 2599-2601 (1980), J. Zimowski, Phytochemistry 30: 1827-1831 (1991)). Stapleton *et al.*, 1991, supra, reported a 600-fold purification of a monomeric, 36- to 38-kilodalton (kDa), soluble protein, SGT from potato sprouts. SGT was isolated by anion-exchange ("Mono Q"), size exclusion ("Superose" 12), and chromatofocusing ("Mono P"). This purification protocol resulted in a very low yield of SGT. A major difficulty encountered was the copurification of SGT with patatin. Patatin is an approximately 40-kDa glycoprotein which can constitute up to 40% of the soluble potato tuber protein.

SUMMARY OF THE INVENTION

The present invention comprises DNA sequences in isolated and purified form which encode the enzyme solanidine UDP-glucose glucosyltransferase (SGT). DNA sequences which hybridize specifically to a SGT coding sequence or its complement under stringent conditions are also encompassed by the present invention. Methods to obtain the sequences are also disclosed herein.

A further aspect of the invention is the provision of recombinant DNA molecules containing the sequences. Such molecules include, for example, recombinant vectors, such as cloning or expression vectors, which contain a DNA sequence encoding SGT.

Another aspect of the invention is the provision of cells which are transformed by the above vectors or DNA sequences.

A further aspect of the present invention is provision of antisense DNA sequences which are capable of being transcribed to form RNA to inhibit the
production of SGT.

A particular use of the invention is the provision of plants or plant cells transformed with an antisense nucleotide sequence complementary to an mRNA-encoding SGT, to provide plants having reduced levels of glycoalkaloids.

A still further aspect of the invention is the provision of oligonucleotide probes capable of detecting the gene for SGT or fragment thereof and use of the probes to isolate DNA sequences encoding SGT. The DNA sequences which hybridize to the probes are encompassed by the present invention.

Another aspect of the invention is the provision of methods to obtain purified SGT. SGT is present in potato cultivars in an extremely low level, is inherently unstable, and copurifies with the major storage protein during isolation.

The invention represents the first successful cloning of SGT. One of the primary advantages of the invention is that it can provide a method to reduce toxic glycoalkaloid concentrations in solanaceous species. Such a method offers a wide variety of benefits extending from the farm, to processing, shipping, and finally to marketing of potatoes and potato products. The ability to reduce toxicant levels in selected varieties will allow introduction of new potato cultivars which cannot currently be released due to glycoalkaloid concentrations exceeding the acceptable level. The utilization of direct genetic modification is especially important to avoid problems of classic potato breeding programs. The genome of commercial potato cultivars grown in the United States (which are tetraploid and highly

heterozygous) is exceedingly complex. This genetic complexity makes it essentially impossible for breeders to introduce a single genetic trait into an existing cultivar, while maintaining its original properties. The invention provides a means to insert a sense or antisense SGT transgene into the genome of these cultivars without altering the existing genes.

Another advantage of the invention is that it provides a means of solving problems in potato storage and shipping due to glycoalkaloids. Inappropriate post-harvest handling of tubers can result in increased glycoalkaloid biosynthesis in current commercial cultivars. The inactivation of glycoalkaloid biosynthetic pathways is beneficial to reduce or eliminate glycoalkaloid biosynthesis during storage and shipping.

Other objects and advantages of this invention will become readily apparent from the ensuing description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a simplified biochemical pathway depicting SGT-catalyzed UDP-glucose glucose dependent glucosylation of solanidine to produce γ-chaconine and biosynthesis of the glycoalkaloids α-chaconine and α-solanine.

FIG. 2 shows the nucleotide and deduced amino acid sequence of the potato SGT cDNA clone.

FIG. 3 shows a comparison of the amino acid sequences of the amino terminal domains of UDP-glycosyltransferases.

FIG. 4 shows a comparison of the amino acid sequences of the UDP binding domain of UDP-glycosyltransferases.

FIG. 5 shows a comparison of a putative steroid-binding domain of three steroid-specific mammalian UDP-glucuronosyltransferases and potato SGT (StSGT).

FIGS. 6A and 6B show the SGT activity of recombinant SGT reaction products in the presence of (A) UDP-[^{3}H-glucose] and (B) ^{3}H-dihydrosolasodine.

FIG. 7 shows the plasmid pA-SGT containing antisense SGT and the GBSS promoter.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides DNA sequences, in purified and isolated form, which encode SGT. For purposes of this invention, the term SGT (solanidine UDP-glucose glucosyltransferase) is defined to mean the enzyme which carries out the UDP-glucose dependent conversion of the aglycone solanidine to γ-chaconine. Because SGT has the potential to play an important role in the regulation of glycoalkaloid accumulation in potato tubers, the partially purified enzyme has been characterized by a number of laboratories (Stapleton *et al.*, 1991, supra, and Stapleton *et al.*, Prot. Expr. Purif. 3:85-92 (1992); Bergenstråhle *et al.*, 1992, supra,; Packowski and Wojciechowski, 1994, supra; Zimowski, 1990, supra). However, purification of SGT to a degree that would allow sequence analysis and cloning has proved to be an elusive goal (Stapleton *et al.*,1992, supra).

The present invention also encompasses methods to obtain isolated DNA sequences having the characteristics described above. As discussed in detail below in the Examples, one method is to clone potato SGT by selection in yeast. Yeast expression libraries have been used to isolate cDNAs encoding a wide variety of plant enzymes employing strategies based either upon complementation (Dewey *et al.*, Plant Cell 6:1495-1507 (1994); Bassham *et al.*, Proc. Natl. Acad. Sci. USA 92:7262-7266 (1995)), screening (Corey *et al.*, Proc. Natl. Acad. Sci. USA 90:11628-11632

(1993)) or selection on growth-inhibiting compounds (Kushnir *et al.*, Proc. Natl. Acad. Sci. 92:10580-10584 (1995)). The selection used here was based on the differential toxicity of aglycons and associated glycosylated forms (Roddick, Phytochemistry 13:9-15 (1974)). A cDNA encoding SGT was selected from a yeast expression library using a positive selection based on the higher toxicity of steroidal alkaloid aglycons relative to their associated glycosylated forms.

The identity of this cDNA as encoding SGT is established both by sequence similarity to previously described UDP-glycosyltransferases and by the characterization of the activity of the recombinant enzyme in yeast. The highly conserved nature of the UDP-glucose binding domain among evolutionarily divergent glycosyltransferases (Hundle *et al.*, Proc. Natl. Acad. Sci USA 89:9321-9325 (1992); Yadav and Brew, J. Biol. Chem. 265:14163-14169 (1990)) allows unambiguous assignment to this family of the enzyme encoded by the cDNA described here (Fig. 4). In addition, the amino terminal domain of potato SGT shares significant similarity with previously described plant glycosyltransferases (FIG. 3). Perhaps the most unexpected result of the database comparisons (Altschul *et al.*, J. Mol. Biol. 215:403-410 (1990)) carried out with this clone was the identification of a putative steroid-binding domain in the deduced amino acid sequence. In contrast to the amino terminal and UDP-glucose domains which preferentially identified glycosyltransferases from plants, only mammalian glucuronosyltransferases were returned after computational searching the available database with the 34 residue peptide shown in Figure 5. Of the returned loci for which substrates are defined, the high-scoring matches represent domains from steroid-specific enzymes.

The availability of the cDNA encoding potato SGT makes accessible both the genomic sequence which provides SGT and closely related enzymes found in the same plant, i.e., potato, as well as other cDNAs encoding SGT from other solanaceous plants. The cDNAs or portions thereof are used as probes to hybridize to the additional genomic or cDNA sequences by hybridization under stringent conditions. Sequences which hybridize specifically to a SGT coding sequence or its complement under stringent conditions are encompassed by the invention. For the purposes of this invention, stringent conditions are defined to mean that hybridization is due to at least about 70% homology, as opposed to nonspecific binding. Homology is defined to mean that the nucleotides match over the defined length of a selected region. Stringent conditions are described in T. Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1982 and DNA Cloning: A Practical Approach, Volumes I and II (Ed. D. N. Glover) IRL Press, Oxford, 1985.

A DNA coding sequence of SGT can also be prepared synthetically from overlapping oligonucleotides whose sequence contains codons for the amino acid sequence of SGT. Such oligonucleotides are prepared by standard methods and assembled and used to isolate the desired SGT gene.

Isolated DNA sequences which encode SGT may also be obtained by hybridization using an oligonucleotide probe capable of detecting a nucleotide sequence which codes for SGT. Such oligonucleotides are prepared by standard methods and assembled by procedures known to those is the art. The length of the probe employed must be sufficient to hybridize to homologous regions of DNA under stringent conditions. Generally it is recognized in the art that probes from about 17 to about 20 base pairs are of

sufficient length to identify homologous sequences. Probes greater than 20 base pairs are more effective.

A DNA sequence which encodes SGT can be used to prepare recombinant DNA molecules (constructs) containing a sequence which encodes SGT, for example, recombinant vectors, such as cloning or expression vectors. A recombinant DNA molecule is prepared by cloning a DNA sequence which encodes SGT into any suitable vector that is capable of introducing a foreign gene into a heterologous host such as a bacterium, a yeast, a virus or its host organism, or in plants.

When the recombinant DNA molecule contains the DNA sequence encoding SGT or a fragment thereof in a 3' to 5' orientation under the control of a suitable promoter such as the Cauliflower Mosaic Virus 35S promoter, the construction is referred to as an antisense transgene. Such transgenes are introduced into hosts by various means, including electroporation, biolistic particle delivery systems, microinjection, transformation, or transfection. Efficacy of the introduced construct is determined by measuring immediate effects produced by transient expression of the introduced molecule or, as is the case in transgenic plants, by DNA-mediated expression of antisense constructs which have been stably introduced into the genome of the host. Suppression of SGT activity lowers the level of glycoalkaloids in plants which have been transformed with an antisense transgene.

Methods of inhibiting SGT production in a cell which normally produces SGT are also encompassed by the invention. For example, as discussed above and in detail below, one method is to modify the cell to contain an antisense nucleotide sequence complementary to an mRNA-encoding SGT. Plants or plant cells transformed with

an antisense nucleotide sequence complementary to an mRNA-encoding SGT have reduced levels of toxic glycoalkaloids.

Isolation and purification of SGT presented problems of unusual difficulty. This enzyme is present at an extremely low level. The amount of SGT in SGT-enriched tissue is only about 0.001% of total protein. Other difficulties were present because SGT is inherently unstable and copurifies with the major storage protein patatin during isolation. Earlier attempts (Stapleton et al., 1991, supra) to isolate SGT were hampered by the small amounts of enzyme present in potato tissue and by the enzyme's instability during purification.

While we (Stapleton *et al.*, 1991 and 1992, supra) and others (Bergenstråhle *et al.* 1992, supra; Packowski and Wojciechowski, 1994, supra; Zimowski, 1990, supra) have reported partial purification of SGT from Solanaceous plants, attempts at purification to an extent that allows sequence analysis and cloning of appropriate cDNAs have so far proved unsuccessful. For this reason we employed an alternative cloning strategy based upon selection in yeast to isolate an SGT encoding cDNA from potato.

Differential toxicity of solanaceous alkaloid aglycons and their associated glycosides has been reported in both mammals (Osman, Phytochemistry 19:2599-2601 (1980)) and fungi (Roddick, 1974, supra). Characterization of the sensitivity of *Saccharomyces cerevisiae* to these secondary metabolites revealed significantly greater toxicity of the aglycons. Although the aglycons screened showed different levels of toxicity (tomatidine>solasodine>solanidine), glycosylated derivatives were considerably less toxic. The differential toxicity in yeast of the aglycones and glycosylated forms was used to set up a positive selection for SGT in this heterologous system as described below.

The synthesis of glycoalkaloids of potato is known to involve a complex series of reactions and interactions that is incompletely understood. A number of enzymes including SGT are thought to be involved in the biosynthetic pathway. It is not clear to what extent the control of only one of these enzymes would be successful in controlling glycoalkaloid biosynthesis. Thus, it was not known to what extent the control of SGT production, taken alone, would be adequate to control the biosynthesis of glycoalkaloids.

However, as illustrated in Example 3 and Table 1, below, control of SGT reduced glycoalkaloid content in transgenic plants.

Definitions

Solanidine UDP-glucose glycosyltransferase (SGT)

As defined herein, "SGT" includes all enzymes which are capable of catalyzing the UDP-glucose dependent conversion of the aglycone solanidine to γ-chaconine. The amino acid sequence of the enzyme may or may not be identical with the amino acid sequence which occurs naturally in solanaceous plants. In addition, artificially induced mutations are also included so long as they do not destroy activity. The definition of SGT used herein includes these variants which are derived by direct or indirect manipulation of the disclosed sequences.

It is also understood that the primary structure may be altered by post-translational processing or by subsequent chemical manipulation to result in a derivatized protein which contains, for example, glycosylation substituents, oxidized forms of, for example, cysteine or proline, conjugation to additional moieties, such as carriers, solid supports, and the like. These alterations

do not remove the protein from the definition of SGT so long as its capacity to catalyze the UDP-glucose dependent conversion of the aglycone solanidine to γ-chaconine is maintained.

Thus, the identity of an enzyme as "SGT" can be confirmed by its ability to carry out SGT enzyme activity. Such an assay is described in Example 2, below.

While alternative forms of assessment of SGT can be devised, and variations on the above protocol are certainly permissible, the foregoing provides a definite criterion for the presence of SGT activity and classification of a test protein as SGT.

Preferred forms of SGT of the invention include those illustrated herein and those derivable by systematic mutation of the genes. Such systematic mutation may be desirable to enhance the SGT properties of the enzyme, to enhance the characteristics of the enzyme which are ancillary to its activity, such as stability, or shelf life, or may be desirable to provide inactive forms useful in the control of SGT activity *in vivo*, as further described below.

As described above, "SGT" refers to a protein having the activity assessed by the assay set forth below; a "mutated SGT" refers to a protein which does not necessarily have this activity, but which is derived by mutation of a DNA encoding an SGT. By "derived from mutation" is meant both direct physical derivation from a DNA encoding the starting material SGT using, for example, site specific mutagenesis or indirect derivation by synthesis of DNA having a sequence related to, but deliberately different from, that of the SGT. As means for constructing oligonucleotides of the required length are available, such DNAs can be constructed wholly or partially from their individual constituent nucleotides.

SGT DNA Coding Sequences

SGT DNA coding sequence includes all DNA sequences in purified and isolated form which encode a SGT meeting the above definition. DNA sequences which hybridize specifically to a SGT coding sequence or its complement under stringent conditions are also encompassed by the present invention. As discussed above, stringent conditions are defined to mean that hybridization is due to at least about 70% homology over a selected region, as opposed to nonspecific binding. For encoding SGT, the sequences should have 70% homology over 500 base pairs, preferably 70% homology over 700 base pairs, and more preferably 70% homology over 1000 base pairs.

Recombinant DNA Molecules

As used herein, "recombinant" refers to a nucleic acid sequence which has been obtained by manipulation of genetic material using restriction enzymes, ligases, and similar recombinant techniques as described by, for example, T. Maniatis et al.,1982, supra, and DNA Cloning: A Practical Approach, 1985, supra. "Recombinant," as used in the present application, does not refer to naturally-occurring genetic recombinations.

A recombinant DNA molecule refers to a hybrid DNA sequence comprising at least two DNA sequences, the first sequence not normally being found together in nature with the second. Examples include recombinant vectors, such as cloning or expression vectors which contain a DNA sequence encoding SGT which is in a 5' to 3' (sense) orientation or in a 3' to 5' (antisense) orientation. Example 1, below, describes preparation of an SGT recombinant DNA molecule.

The DNA sequences of the invention are also useful to prepare recombinant DNA expression molecules by cloning the sequence in any suitable expression vector using known techniques. The recombinant vector is constructed so that the coding sequence is located in the vector with the appropriate control sequence and operationally associated therewith, that is, the positioning and orientation of the SGT DNA coding sequence with respect to the control sequences are such that the coding sequence is transcribed under the control of the control sequences (i.e., by RNA polymerase which attaches to the DNA molecule at the control sequences). The control sequences may be ligated to the coding sequence prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequence and an appropriate restriction site downstream from the control sequence. The vector should be selected so as to have a promoter operable in the host cell into which the vector is to be inserted (that is, the promoter should be recognized by the RNA polymerase of the host cell).

Expression Systems

A recombinant DNA expression molecule containing a sequence which encodes SGT in the 5' to 3' orientation is inserted into a host cell for expression of SGT. A variety of expression systems and hosts are known in the art for production of an enzyme. Examples of prokaryotic hosts are *Escherichia coli* and other bacterial hosts such as *B. subtilis* or *Pseudomonas*. Typical bacterial promoters include the trp, lac, tac, and ß-lactamase

promoters. A large number of recombinant systems have been developed for expression in eukaryotic hosts, including yeast, insect cells, mammalian cells, and plant cells. These systems are well characterized, and require the ligation of the coding sequence under the control of a suitable transcription initiating system (promoter) and, if desired, termination sequences and enhancers. For production of SGT, host cells transformed by a recombinant DNA expression molecule are grown, and the protein isolated from the host cells. The selection of appropriate growth conditions and recovery methods are within the skill of the art.

For expression in yeast, a library of wound-induced potato tuber cDNA was introduced and the yeast transformants were selected on solasodine-containing medium. Resistance to the alkaloid indicated the presence of SGT.

The coding sequence for SGT and the DNA which represents the reverse transcript of the mRNA that is subsequently translated into SGT can be included in expression systems suitable for plants.

Transformation of solanaceous plants which normally produce SGT, e.g., potato and tomato, with a recombinant expression system for the relevant SGT or a truncated form thereof may result, through an unknown mechanism, in suppression of the native production of SGT, and may thus provide a means to inhibit, for example, the biosynthesis of glycoalkaloids in such plants. This phenomenon has been referred to as "cosuppression". It has been shown previously that attempts to overexpress chalcone synthase in pigmented petunia petals by introducing the recombinant gene resulted in a suppression of the homologous native genes, thus resulting in a block in biosynthesis (C. Napoli *et al.*, The Plant Cell 2: 279-289 (1990)). These results were confirmed and

extended to transformation with genes encoding dihydroflavonol-4-reductase genes in petunias by A.R. van der Krol *et al.*, The Plant Cell 2: 291-299 (1990). It has also been found that transformation of a partial nopaline synthase gene into tobacco suppresses the expression of the endogenous corresponding gene, as reported by D.R. Goring *et al.*, Proc. Natl. Acad. Sci. USA 88: 1770-1774 (1991). In general, it appears that supplying a truncated form of the relevant gene in the "sense" orientation suppresses the endogenous expression of the native gene, thus lowering the level of the gene product, despite the presence of the additional expressed gene coding sequences.

Alternatively, a DNA which is transcribed into the complement of mRNA that is translated by the host plant into SGT can be provided to effect an antisense retardation of expression of the native gene.

Especially useful in connection with the SGT genes of the present invention are expression systems which are operable in plants. These include systems which are under control of a tissue-specific promoter, as well as those which involve promoters that are operable in all plant tissues.

Transcription initiation regions, for example, include the various opine initiation regions, such as octopine, mannopine, nopaline and the like. Plant viral promoters can also be used, such as the cauliflower mosaic virus 35S promoter. In addition, plant promoters such as ribulose-1,3-diphosphate carboxylase, fruit-specific promoters, heat shock promoters, seed-specific promoters, etc. can also be used.

The cauliflower mosaic virus (CaMV) 35S promoter has been shown to be highly active in many plant organs and during many

stages of development when integrated into the genome of transgenic plants.

Organ-specific promoters are also well known. For example, the patatin class 1 promoter is transcriptionally activated only in the potato tuber and can be used to target gene expression in the tuber (M. Bevan, Nucleic Acids Research 14: 4625-4636 (1986)). The granule-bound starch synthase (GBSS) promoter is also a potato-specific promoter (R.G.R. Visser *et al*, Plant Molecular Biology 17:691-699 (1991)).

Other organ-specific promoters appropriate for a desired target organ can be isolated using known procedures. These control sequences are generally associated with genes uniquely expressed in the desired organ. In a typical higher plant, each organ has thousands of mRNAs that are absent from other organ systems (reviewed in P. Goldberg, Trans R Soc London B314: 343 (1986)).

For expression in plants, the recombinant expression cassette will contain in addition to the SGT, a plant promoter region, a transcription initiation site (if the coding sequence to transcribed lacks one), and a transcription termination/polyadenylation sequence. The termination/polyadenylation region may be obtained from the same gene as the promoter sequence or may be obtained from different genes. Unique restriction enzyme sites at the 5' and 3' ends of the cassette are typically included to allow for easy insertion into a pre-existing vector.

For *in situ* production of the antisense mRNA of SGT, those regions of the SGT gene which are transcribed into SGT mRNA, including the untranslated regions thereof, are inserted into the expression vector under control of the promoter system in a

reverse orientation. The resulting transcribed mRNA is then complementary to that normally produced by the plant. The presence of the antisense mRNA, as shown in Table 1, effectively reduces glycoalkaloid biosynthesis.

TABLE 1

Effect of Antisense Solanidine Glucosyltransferase Expression on Tuber Glycoalkaloid Levels in Transgenic Potato Clones

Sample	Total Glycoalkaloid * (Aberdeen)	% Wild Type (Aberdeen)	Total Glycoalkaloid * (Albany)	% Wild Type (Albany)
Wild Type (Lenape)	79	100	164	100
Len35S#7	57	72	73	44
Len35S#8	32	40	NA	NA
LenGBSS#6	87	109	84	51
LenGBSS#10	43	54	NA	NA
LenGBSS#11	32	41	56	33
LenGBSS#12	22	28	NA	NA

*mg/100g fresh wt.

Table 1 shows the glycoalkaloid content in potato tubers from transgenic potato clones expressing antisense solanidine glucosyltransferase (SGT) mRNA. Two SGT antisense cassettes were employed, in which the antisense RNA was transcribed from either a Cauliflower Mosaic Virus 35S (35S) promoter or a tuber-specific Granule Bound Starch Synthase (GBSS) promoter. These constructs

were introduced into the potato cultivar Lenape and greenhouse-grown mini-tubers were analyzed at Aberdeen, ID (Aberdeen) or at Albany, CA (Albany). The standard colorometric assay (Fitzpatrick *et al.*, American Potato Journal 51:318-323 (1974)) was used at Aberdeen and a HPLC based method (Friedman *et al.*, J Agric Food Chem 40:2157-2163 (1992)) was employed in Albany.

The resulting expression system or cassette is ligated into or otherwise constructed to be included in a recombinant vector which is appropriate for plant transformation. The vector will also typically contain a selectable marker gene by which transformed plant cells can be identified in culture. Usually, the marker gene will encode antibiotic resistance. These markers include resistance to G418, hygromycin, bleomycin, kanamycin, and gentamicin. After transforming the plant cells, those cells having the vector will be identified by their ability to grow on a medium containing the particular antibiotic. Replication sequences, of bacterial or viral origin, are generally also included to allow the vector to be cloned in a bacterial or phage host, preferably a broad host range prokaryotic origin of replication is included. A selectable marker for bacteria should also be included to allow selection of bacterial cells bearing the desired construct. Suitable prokaryotic selectable markers also include resistance to antibiotics such as kanamycin or tetracycline.

Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art. For instance, in the case of *Agrobacterium* transformations, T-DNA sequences will also be included for subsequent transfer to plant chromosomes.

In addition, vectors can also be constructed that contain in-frame ligations between the sequence encoding the SGT protein

and sequences encoding other molecules of interest resulting in fusion proteins, by techniques well known in the art.

When an appropriate vector is obtained, transgenic plants are prepared which contain the desired expression system. A number of techniques are known in the art for transformation of plants or plant cells.

For transformation mediated by bacterial infection, a plant cell is infected with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the DNA to be introduced. *Agrobacterium* is a representative genus of the gram-negative family Rhizobiaceae. Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome (J. Schell, Science 237: 1176-1183 (1987)). Ti and Ri plasmids contain two regions essential for the production of transformed cells.

Construction of recombinant Ti and Ri plasmids in general follows methods typically used with the more common bacterial vectors, such as pUC19. There are two classes of recombinant Ti and Ri plasmid vector systems now in use. In one class, called "cointegrate," the shuttle vector containing the gene of interest is inserted by genetic recombination into a non-oncogenic Ti plasmid that contains both the cis-acting and trans-acting elements required for plant transformation as, for example, in the pMLJ1 shuttle vector of DeBlock *et al.* (EMBO J 3: 1681-1689 (1984)) and the non-oncogenic Ti plasmid pGV3850 described by Zambryski *et al.* (EMBO J 2: 2143-2150 (1983)). In the second class or "binary" system, the gene of interest is inserted into

a shuttle vector containing the cis-acting elements required for plant transformation. The other necessary functions are provided in trans by the non-oncogenic Ti plasmid as exemplified by the pBIN19 shuttle vector described by Bevan (Nucleic Acids Research 12: 8711-8721 (1984)) and the non-oncogenic Ti plasmid PAL4404 described by Hoekema *et al.* (Nature 303: 179-180 (1983)). Some of these vectors are commercially available.

There are two common ways to transform plant cells with *Agrobacterium*: co-cultivation of *Agrobacterium* with cultured isolated protoplasts, or transformation of intact cells or tissues with *Agrobacterium*. The first requires an established culture system that allows for culturing protoplasts and subsequent plant regeneration from cultured protoplasts. The second method requires (a) that the intact plant tissues, such as cotyledons or potato tuber discs, can be transformed by *Agrobacterium* and (b) that the transformed cells or tissues can be induced to regenerate into whole plants. Most dicot species can be transformed by *Agrobacterium* as all species which are a natural plant host for *Agrobacterium* are transformable *in vitro*.

Identification of transformed cells or plants is generally accomplished by including a selectable marker in the transforming vector, or by obtaining evidence of successful bacterial infection.

Plant cells which have been transformed can be regenerated using known techniques. Plant regeneration from cultured protoplasts is described in Evans *et al.*, Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co. New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. II, 1986).

It is known that practically all plants can be regenerated from cultured cells or tissues.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, somatic embryo formation can be induced in the callus tissue. These somatic embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and plant hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable. The regenerated plants are transferred to standard soil conditions and cultivated in a conventional manner.

After the expression cassette is stably incorporated into regenerated transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed. The plants are grown and harvested using conventional procedures.

Antisense Expression

When the SGT gene or a fragment thereof is cloned into a heterologous expression vector in the opposite orientation (e.g. in the 3' to 5' orientation) to that found in nature, an antisense construct is created. Transcription of the antisense region of such a construct results in the production of an RNA molecule

complementary to the messenger RNA (mRNA) of the gene or genes being targeted. The activity of such a construct in either transient or stable DNA-mediated transformation systems results in the inhibition of SGT activity with subsequent reduction of SGT activity or glycoalkaloid levels in the plant or system containing the construct. The antisense SGT portion of the construct must be of sufficient size to provide the desired inhibitory effect. As few as 52 bases of 5' untranslated antisense RNA has been shown to inhibit enzymatic activity in some systems (J. G. Izant and H. Weitraub, Science 229: 345-352 (1985)). The sequence complementary to a sequence of the messenger RNA will usually be at least 50 nucleotides, preferably about 100 nucleotides or more and may include the entire length of the coding region.

Similarly, a gene or a fragment thereof or multiple copies of the fragments can be cloned into a heterologous expression system in the native orientation and cause co-suppression or inhibition of an indigenous enzyme (C. Napoli, C. Lemieux, and R. Jorgensen, The Plant Cell 2: 279-289 (1990); C. J. S. Smite *et al.*, Molec. Gen. Genet. 224: 477-481 (1990); and A. R. van der Krol *et al.*, The Plant Cell 2: 291-299 (1990)).

The antisense constructs are useful to inhibit SGT activity in plants which normally produce SGT, e.g., potato and tomato, and thereby reduce glycoalkaloid levels. Example 3, below, illustrates that glycoalkaloid biosynthesis in potatoes can be controlled and inhibited by antisense expression of the SGT coding sequence supplied in a construct under the control of the Cauliflower Mosaic Virus 35S and GBSS promoters.

Numerous issued U.S. Patents are available which disclose information useful to those skilled in the art in practicing the present invention. U.S. Patent No. 4,710,463 to Murray discloses

recombinant DNA expression vectors incorporating DNA sequences coding for a foreign polypeptide, e.g., Hepatitis B virus antigens, in a unicellular host. U.S. Patent No. 4,440,859 to Rutter *et al.* discloses recombinant bacterial plasmids containing the coding sequences of higher organisms. U.S. Patent No. 4,652,525 to Rutter *et al.* discloses isolation of a nucleotide coding sequence having the structure of the reverse transcript of an mRNA which encodes insulin, synthesis of double stranded DNA having the sequence, and transfer of the DNA to a host microorganism. U.S. Patent No. 4,546,082 to Kurjan and Herskowitz discloses methods for expression of biologically useful heterologous polypeptides in yeast. U.S. Patent No. 4,582,800 to Crowl discloses expression vectors which utilize transcriptional regulatory elements derived from bacteriophage lambda. U.S. Patent No. 4,363,877 to Goodman and Seeburg discloses isolation of cDNA transcripts complementary to isolated mRNA, and recombinant DNA vectors containing codons for human somatomammotropin and for human growth hormone. U.S. Patent No. 4,601,980 to Goeddel and Heyneker discloses the expression of a gene coding for human growth hormone in a pBR322/*E. coli* system. U.S. Patent No. 4,590,163 to Helinski and Ditta discloses PK2 plasmids useful for gene cloning in gram-negative bacteria such as *E. coli*. U.S. Patent No. 4,237,224 to Cohen and Boyer discloses methods for producing recombinant DNA expression vectors. U.S. Patent Nos. 4,468,464 and 4,740,470 to Cohen and Boyer describe biologically functional molecular chimeras. U.S. Patent No. 4,940,838 to Schilperoort and Hoekema describes a process for the incorporation of foreign DNA Into the genome of dicotyledonous plants. U.S. Patent No. 4,332,897 to Nakano *et al.* discloses lamboid bacteriophage vectors useful for transforming

E. coli. U.S. Patent No. 4,332,901 to Goldstein discloses a P4 derivative bacteriophage cloning vector. U.S. Patent No. 4,704,362 to Itakura and Riggs and U.S. Patent No. 4,356,270 to Itakura disclose recombinant plasmid vectors useful for transforming microbial hosts. U.S. Patent No. 4,273,875 to Manis discloses a plasmid designated PUC6 useful as a cloning vector for transforming microbial hosts. U.S. Patent No. 4,349,629 to Carey *et al*. discloses plasmid vectors employing the trp bacterial promoter useful as recombinant DNA expression vectors. U.S. Patent No. 4,362,817 to Reusser discloses the plasmid pUC1060, which contains a tet gene promoter, useful as an expression vector. U.S. Patent Nos. 4,565,785 and 4,411,994 by Gilbert *et al*. discloses a recombinant DNA molecule having a bacterial gene and non-bacterial gene encoding a selected polypeptide. U.S. Patent No. 4,683,195 by Mullin describes a process for amplifying nucleic acid sequences. U.S. Patent No. 4,801,540 by Hiatt *et al*. discloses a DNA sequence encoding polygalacturonase (PG) and its use to modulate PG expression in plant cells. U.S. Patent No. 5,107,065 by Shewmaker *et al*. discloses antisense regulation of gene expression in plant cells. U.S. Patent No. 5, 168,064 by Bennett *et al*. describes a method of inhibiting endo-1,4-b-glucanase activity in plants using antisense DNA constructions. U.S. Patent No. 5,073,676 to Bridges *et al*. describes tomato antisense pectin esterase. U.S. Patent 5,034,323 by Jorgensen *et al*. describes a method for altering color patterns of flowers and other plant parts. The disclosures of all U.S. patent references cited herein are to be incorporated herein by reference.

EXAMPLES

The following examples are intended only to further illustrate the invention and are not intended to limit the scope of the invention which is described by the claims.

EXAMPLE 1

Selection of SGT cDNA in yeast

A yeast expression vector library containing cDNAs from wounded tuber tissue was constructed in *E. coli*. Yeast (*Saccharomyces cerevisiae*) strain KT1115 (*MATα, leu2-3, leu2-112, ura3-52*) (Dewey *et al.*, Plant Cell 6:1495-1507 (1994)) was cultured on YPD medium (Sherman, Methods Enzymol. 194:3-20 (1991)). Growth of KT1115 on SGAs and associated aglycons was assessed by plating the strain on YPGal (YP-2% galactose) medium incorporating various dilutions of alkaloids or SGAs prepared as 1-10mM stock solutions in ethanol. Solanidine, solasodine, tomatidine and α-chaconine were purchased from Sigma. Gamma chaconine was prepared by partial acid hydrolyis of α-chaconine and HPLC purification as described by Friedman *et al.* (J. Agric. Food Chem. 41:1397-1406 (1993)).

A yeast expression library of wound-induced potato tuber cDNAs was prepared by excising cDNA inserts from a previously constructed λgt11 library (Garbarino *et al.*, Plant Mol. Biol. 20:235-244 (1992)) with BamH1 and ligating them into the pYES2 expression vector (Invitrogen). The ligation mixture was transformed into *E. coli* strain DH5α by electroporation. Twenty random clones were analyzed by restriction digestion to confirm the presence of inserts. In the pYES2 expression vector employed, introduced sequences are transcribed from a *gal1* galactose-inducible promoter. The library was introduced into KT1115 as

described by Gietz *et al.* (Nucl. Acids Res. 20:1425 (1992)) and selected on minimal medium lacking uracil. Yeast transformants (>10,000) were then replica plated onto galactose containing medium with or without 50 μM solasodine. After one week, colonies growing on the solasodine-containing medium were transferred to minimal medium lacking uracil to ensure maintenance of the plasmid. Four of the approximately 10^4 colonies screened were selected for analysis based upon apparent growth in the presence of the alkaloid. Plasmid DNA was prepared from the yeast (Strathern and Higgins, Methods Enzymol. 194:302-318 (1991)) and transformed into *E. coli*. Ability to confer resistance to solasodine toxicity was confirmed by re-transformation of KT1115 following amplification in *E. coli*. Following transformation into KT1115, only one of the plasmids was found to confer resistance to the alkaloid. The observed resistance was dependent upon the presence of galactose as a carbon source.

Sequence analysis of potato SGT

The selected cDNA, designated StSGT, was 1578 bp in length and contained an open reading frame encoding a 483 residue polypeptide that appeared to lack an initiator methionine (FIG. 2). In order to obtain additional 5' sequence, a potato tuber λgt11 library (Garbarino *et al.*, 1992, supra) was used as a template in PCR amplification with one StSGT specific primer and a second primer located within the λ vector (FIG. 2). Inserts from positive pYES2 clones were subcloned into pUC19 and sequenced using the fmol DNA sequencing system of Promega™. Sequence analysis of the longest clone resulted in 34 bp of additional sequence 5' to the original StSGT isolate that encoded a potential initiator methionine (FIG.

2). The shaded deduced amino acid sequences indicate conserved domains associated with the amino terminus (see FIG. 3), the putative steroid binding site (see FIG. 5) and the UDP-glucose binding site (see FIG. 4). The arrow indicates the 5' end of the original StSGT cDNA clone. Additional 5' sequence was obtained using a primer complementary to the shaded DNA sequence.

A database search (Altschul *et al.*, 1990, supra) with the deduced StSGT coding sequence revealed significant similarity to previously characterized UDP-glycosyltransferases from both eukaryotic and prokaryotic species. FIG. 3 shows a comparison of the amino acid sequences of the amino terminal domains of UDP-glycosyltransferases. The deduced amino acid sequence of potato SGT (StSGT) is compared to amino terminal sequences of an anthocyanidin UDP-rhamnosyltransferase from petunia (PhART, GenBank assession #S33169), a maize IAA glucosyltransferase (IAAGT, GenBank assession #A54739) and a rhamnosyltransferase from *Pseudomonas aerugenosa* (ParRT, GenBank locus B53652). Numbers indicate position relative to the putative initiator methionine. Residues highlighted in black indicate positions of identity. Shaded residues indicate conservative substitutions. FIG. 4 shows a comparison of the amino acid sequences of the UDP binding domain of UDP-glycosyltransferases. The deduced amino acid sequence of potato SGT (StSGT) is compared to sequences of a UDP-glucosyltransferase from cassava (CasGT, GenBank assession #S41951), IAAGT and PhART as in FIG. 3, and maize bronze-1 (MzBz1, GenBank Locus UF03). Numbering, highlighting and alignments were determined as in FIG. 3. FIG. 5 shows a comparison of a putative steroid-binding domain of three steroid-specific mammalian UDP-glucuronosyltranferases and potato SGT (StSGT). The deduced amino acid sequence of SGT is compared to sequences of human 3,4-

catechol estrogen-specific (Hudb7, GenBank assession #P16662) and polyhydroxylated estrogen-specific (Hudbb, GenBank assession #P36538) enzymes, and a rat β-hydroxysteroid UDP-glucuronosyltransferase (Rudb6, GenBank assession #P19488). Numbering, highlighting and alignments were determined as in FIG. 3. Regions of high similarity to other plant UDP-glycosyltransferases were observed in both the amino- (FIG. 3) and carboxyl-terminal (FIG. 4) domains of the deduced amino acid sequence. An alignment of the StSGT putative UDP-glucose binding domain (Hundle *et al.*, 1992, supra; Yadav and Brew, 1990, supra) to a selection of plant UDP-glycosyltransferases is shown in FIG. 4. In contrast to these domains, a database search with an internal sequence (residues 109-143) revealed similarity to a series of steroid-specific UDP-glucuronosyltranferases from mammals (FIG. 5), with no significant similarity to previously described plant enzymes. The sequence alignment shown in FIG. 5 suggests that this domain represents a part of a steroid binding domain in these enzymes. The observed similarities of the StSGT deduced amino acid sequence to previously described transferases suggest that this cDNA encodes a UDP-glycosyltransferase that glycosylates steroid-related substrates.

EXAMPLE 2

Recombinant SGT enzyme activity in yeast

Characterization of the recombinant potato SGT in yeast required partial purification to remove competing UDP-glucose hydrolyzing enzymes, also required for analysis of SGT activity in potato tissue. Yeast cells containing pStSGT or pYES2 were grown in liquid culture YNB-Glucose to 0.8 OD_{595}/ml then centrifuged (5.3K x g, 5min, 4°C) to pellet the cells. The pellet

was washed in sterile water, recentrifuged and suspended in 10ml YPA-Galactose media then transferred into 250ml of YPA-Gal and grown to 3.0 OD_{595}/ml. The washed YPA-Gal cells were permeabilized by resuspending the pellet in cold extraction buffer (20mM Bis-Tris propane, pH 7.7, 5mM $MgCl_2$, 1mM DTT, 0.05% Triton X-100, and 0.1mM PMSF), 100mg wet pellet/ml. This material was placed in liquid nitrogen for 10min then held overnight at -80°C (Miozzari, Anal. Biochem. 90:220-233 (1978)). The thawed sample was centrifuged (20K x g, 10min, 4°C), and the resulting supernatant was further purified to evaluate SGT activity.

Characterization of authentic potato SGT in crude extracts indicated the necessity for an initial anion-exchange chromatography purification step prior to SGT activity determinations. This was confirmed with yeast extracts derived from pStSGT containing cells. Lysate supernatants (15mg/30ml) were loaded onto a 5ml BioRad Econo-Q-cartridge pre-equilibrated with 50 ml of extraction buffer (minus Triton X-100 and PMSF). The nonbinding material was washed from the cartridge with 30ml of extraction buffer at a flow rate of 2.0 ml/min. Proteins were eluted with a 0.25M KCl step gradient at a 2.0 ml/min flow rate. One minute fractions were collected and 200 µl aliquots were assayed for enzyme activity using 10 µl of 1 mM of the aglycons solanidine, solasodine, tomatidine in DMSO or DMSO alone with 80 µl of 100mM Bis-Tris propane buffer, pH 6.6, plus 10 µl of UDP-[^{3}H-glucose] (Stapleton *et al.*, 1991, supra).

Characterization of the substrate specificity of SGT purified from potato demonstrated that the endogenous enzyme glucosylates tomatidine and solasodine at rates significantly higher than solanidine (solasodine>tomatidine>solanidine). Similarly, the recombinant enzyme from yeast glucosylated solanidine at a rate

lower than the other two aglycons. However, in contrast to the endogenous enzyme, the recombinant SGT glucosylated tomatidine at a rate greater than was observed for solasodine. No SGT activity was observed in extracts prepared from *S. cerevisiae* containing the empty vector, pYES2, controls.

In order to verify the identity of the recombinant SGT reaction products, samples generated using either UDP-[^{3}H-glucose] or ^{3}H-dihydrosolasodine as substrates were analyzed by TLC (FIG. 6A and 6B). The UDP-[^{3}H-glucose] assays were incubated for 30 minutes as described previously (Stapleton *et al.*, 1991, supra) and the remaining assay supernatant, approximately 200 µl, was lyophilized and extracted two times with 10 µl of methanol and then analyzed by TLC. ^{3}H-dihydrosolasodine was prepared by reduction of solasodine with tritium gas (American Radiolabeled Chemicals Inc., St. Louis, MO). For the ^{3}H-dihydrosolasodine assay, 10mM UDP-glucose was substituted for the UDP-[^{3}H-glucose] and the 300 µl final volume was lyophilized and extracted two times with 10 µl of methanol and then analyzed by TLC. The TLC was performed on Merck precoated silica gel 60 plates in a saturated chamber of chlorform:methanol:2% NH_4OH, 70:30:5. One centimeter sections were scraped from the plates and analyzed by scintillation counting. The plates were then briefly dipped in Calcofluor 0.02%-methanol (Jellema *et al.*, J. Chrom. 176:435-439 (1979)) to determine the distance from the origin, (cm/R_f), for solanidine (9.1/0.83), solasodine (9.3/0.85), and γ-chaconine (5.9/0.54). The radioactive peaks were then compared to the two aglycons and the glycosylated solanidine product.

For both substrates, radiolabeled reaction products migrated to positions indistinguishable from authentic γ-chaconine. Synthesis of these reaction products was dependent upon the

presence of the StSGT cDNA insert and was not observed in the pYES2 controls (FIG. 6A and 6B).

EXAMPLE 3

The following example describes the transformation of potato plants with clone StSGT (1578 bp) in an antisense orientation.

Construction of Antisense SGT Chimeric Plasmids and Use in Transgenic Potatoes

The 1578 bp fragment was excised from plasmid StSGT with the restriction endonuclease BamHI. After purifying the fragment with "Geneclean II" (Bio 101), it was cloned into plasmid pARS201 to form a chimera consisting of the Cauliflower Mosaic Virus 35S promoter or the GBSS promoter fused to the partial SGT sequence in an antisense orientation followed by the nopaline synthase (nos) terminator region from plasmid pBI121 (Bevan *et al.*, Nucleic Acids Research 11: 369-385 (1983). The 35S and GBSS transgenes were subsequently removed intact as a HindIII fragment and cloned into plasmid pCGN1547 (McBride and Summerfelt, Plant Molecular Biology 14: 269-276 (1990)). The restriction map of the resulting SGT binary antisense plasmid pA-SGT is shown in FIG. 7. The plasmid was transformed into *E. coli* strain TB-1 and, after confirming the orientation of the SGT insert, it was transformed into *Agrobacterium tumefasciens* strain PC2760 (An *et al.*, EMBO J. 4: 277-284 (1985)). The binary vector pCGN1547 contains the neomycin phosphotransferase (nptII) gene (Bevan *et al.*, Nucleic Acids Research 12: 8711-8721 (1984)) which confers kanamycin resistance, and expression in the plants is driven by the mannopine synthase promoter. Cultures of PC2760 harboring the modified binary vector pA-SGT were grown at 29°C in liquid

Luria-Bertain (LB) broth supplemented with 20 mg/L gentamycin. Four hours before microtuber inoculation, 50 μM 3',5'-dimethoxy-4'-hydroxyacetophenone (acetosyringone) (Aldrich Chemical Co.) was added to the 50 ml *Agrobacterium* culture.

Transformations

Potato microtubers were transformed with the antisense plasmid using an *Agrobacterium*-mediated procedure described in Snyder *et al.*, Plant Cell Reports, 12:324-327 (1993), which is incorporated herein by reference.

Green-house grown mini-tubers were transformed with antisense plasmids containing either the Cauliflower Mosaic Virus 35S promoter or the Granule Bound Starch Synthase promoter (see Table 1).

Transgenic material is being propagated for planting, and the mature field-grown tubers will be analyzed using either the colorometric or HPLC methods, as described above.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(I) APPLICANT: MOEHS, CHARLES P
ALLEN, PAUL V
ROCKHOLD, DAVID R
STAPLETON, ANDREW
GARBARINO, JOAN E
FRIEDMAN, MENDEL
BELKNAP, WILLIAM R

(ii) TITLE OF INVENTION: DNA SEQUENCES ENCODING SOLANIDINE UDP-GLUCOSE GLUCOSYLTRANSFERASE AND USE TO REDUCE GLYCOALKALOIDS IN SOLANACEOUS PLANTS

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(B) TELEFAX: (510) 559-5777

(2) INFORMATION FOR SEQ ID NO:1:

(I) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1607 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Solanum tuberosum
(B) STRAIN: cv. Lemhi Russet
(C) INDIVIDUAL ISOLATE: SGT1750
(D) DEVELOPMENTAL STAGE: mature, somatic
(F) TISSUE TYPE: tuber

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: lambda gt11 cDNA library
(B) CLONE: SGT 1750

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 20..1486
(D) OTHER INFORMATION: /product= "solanidine glucosyltransferase"

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Moehs, Charles P.
Allen, Paul V.
Friedman, Mendel
Belknap, William R.
(B) TITLE: Cloning and expression of solanidine UDP-glucose glucosyltransferase from potato
(C) JOURNAL: The Plant Journal
(D) VOLUME: 11
(E) ISSUE: 2
(G) DATE: 1997
(K) RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 488

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CTGTTCTTGG GTAGTAAAA ATG GTA GCA ACC TGC AAC AGT GGC GAA ATC CTC        52
                      Met Val Ala Thr Cys Asn Ser Gly Glu Ile Leu
                        1               5                  10

CAT GTT CTT TTC CTT CCC TTC TTA TCC GCT GGT CAT TTC ATC CCA TTA        100
His Val Leu Phe Leu Pro Phe Leu Ser Ala Gly His Phe Ile Pro Leu
            15                  20                  25

GTT AAC GCC GCA AGG CTA TTC GCC TCC CGC GGT GTT AAA GCC ACA ATC        148
Val Asn Ala Ala Arg Leu Phe Ala Ser Arg Gly Val Lys Ala Thr Ile
        30                  35                  40

CTC ACT ACC CCT CAT AAT GCC TTA CTT TTT AGA TCT ACT ATT GAC GAT        196
Leu Thr Thr Pro His Asn Ala Leu Leu Phe Arg Ser Thr Ile Asp Asp
    45                  50                  55
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GAT GTT CGA ATT TCC GGA TTT CCC ATT TCT ATC GTA ACT ATT AAA TTC        244
Asp Val Arg Ile Ser Gly Phe Pro Ile Ser Ile Val Thr Ile Lys Phe
 60                  65                  70                  75

CCC TCT GCT GAA GTT GGG TTG CCT GAA GGA ATT GAG AGC TTT AAC TCT        292
Pro Ser Ala Glu Val Gly Leu Pro Glu Gly Ile Glu Ser Phe Asn Ser
                 80                  85                  90

GCC ACT TCA CCT GAA ATG CCT CAT AAA ATT TTT TAT GCT CTT TCT CTT        340
Ala Thr Ser Pro Glu Met Pro His Lys Ile Phe Tyr Ala Leu Ser Leu
             95                 100                 105

CTA CAA AAG CCA ATG GAA GAT AAA ATT CGT GAA CTC CGT CCT GAT TGC        388
Leu Gln Lys Pro Met Glu Asp Lys Ile Arg Glu Leu Arg Pro Asp Cys
        110                 115                 120

ATT TTT TCT GAT ATG TAC TTC CCT TGG ACA GTA GAT ATT GCT GAT GAG        436
Ile Phe Ser Asp Met Tyr Phe Pro Trp Thr Val Asp Ile Ala Asp Glu
    125                 130                 135

CTT CAC ATC CCT CGT ATT TTG TAC AAT TTG TCT GCT TAC ATG TGC TAC        484
Leu His Ile Pro Arg Ile Leu Tyr Asn Leu Ser Ala Tyr Met Cys Tyr
140                 145                 150                 155

AGC ATT ATG CAC AAC CTT AAG GTT TAC AGA CCT CAC AAG CAG CCT AAT        532
Ser Ile Met His Asn Leu Lys Val Tyr Arg Pro His Lys Gln Pro Asn
                160                 165                 170


CTA GAC GAA TCT CAA AGT TTC GTG GTT CCT GGT TTA CCT GAT GAG ATA        580
Leu Asp Glu Ser Gln Ser Phe Val Val Pro Gly Leu Pro Asp Glu Ile
            175                 180                 185

AAG TTC AAG TTA TCC CAA CTG ACA GAT GAT CTG AGA AAG TCG GAT GAC        628
Lys Phe Lys Leu Ser Gln Leu Thr Asp Asp Leu Arg Lys Ser Asp Asp
        190                 195                 200

CAA AAG ACT GTT TTT GAC GAA TTG CTC GAA CAA GTT GAA GAT TCG GAG        676
Gln Lys Thr Val Phe Asp Glu Leu Leu Glu Gln Val Glu Asp Ser Glu
    205                 210                 215

GAA CGA AGC TAT GGC ATT GTT CAT GAT ACA TTT TAT GAG CTA GAA CCT        724
Glu Arg Ser Tyr Gly Ile Val His Asp Thr Phe Tyr Glu Leu Glu Pro
220                 225                 230                 235

GCA TAT GTT GAC TAC TAC CAG AAA TTA AAG AAA CCA AAA TGT TGG CAT        772
Ala Tyr Val Asp Tyr Tyr Gln Lys Leu Lys Lys Pro Lys Cys Trp His
                240                 245                 250

TTT GGT CCG CTC TCT CAT TTT GCA TCC AAA ATC CGT AGT AAG GAA CTA        820
Phe Gly Pro Leu Ser His Phe Ala Ser Lys Ile Arg Ser Lys Glu Leu
            255                 260                 265
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ATT TCT GAG CAT AAC AAC AAT GAG ATT GTT ATA GAT TGG TTG AAT GCA      868
Ile Ser Glu His Asn Asn Asn Glu Ile Val Ile Asp Trp Leu Asn Ala
        270                 275                 280

CAG AAA CCT AAA TCG GTT CTC TAT GTA TCT TTC GGA AGC ATG GCT AGA      916
Gln Lys Pro Lys Ser Val Leu Tyr Val Ser Phe Gly Ser Met Ala Arg
    285                 290                 295

TTT CCT GAG AGC CAA CTG AAT GAA ATA GCC CAA GCT CTG GAT GCT TCA      964
Phe Pro Glu Ser Gln Leu Asn Glu Ile Ala Gln Ala Leu Asp Ala Ser
300                 305                 310                 315

AAT GTT CCT TTC ATT TTT GTA TTG AGG CCT AAT GAA GAA ACG GCG TCG      1012
Asn Val Pro Phe Ile Phe Val Leu Arg Pro Asn Glu Glu Thr Ala Ser
                320                 325                 330

TGG TTG CCA GTT GGT AAT TTA GAG GAC AAG ACT AAA AAG GGT TTG TAC      1060
Trp Leu Pro Val Gly Asn Leu Glu Asp Lys Thr Lys Lys Gly Leu Tyr
            335                 340                 345

ATC AAA GGG TGG GTC CCA CAG CTT ACG ATC ATG GAA CAT TCA GCA ACA      1108
Ile Lys Gly Trp Val Pro Gln Leu Thr Ile Met Glu His Ser Ala Thr
        350                 355                 360

GGC GGG TTC ATG ACT CAT TGT GGT ACT AAT TCG GTT CTG GAA GCC ATC      1156
Gly Gly Phe Met Thr His Cys Gly Thr Asn Ser Val Leu Glu Ala Ile
    365                 370                 375

ACT TTT GGC GTG CCA ATG ATA ACA TGG CCA CTT TAT GCT GAT CAA TTC      1204
Thr Phe Gly Val Pro Met Ile Thr Trp Pro Leu Tyr Ala Asp Gln Phe
380                 385                 390                 395

TAC AAC GAG AAG GTA GTC GAG GTT AGG GGA TTG GGA ATC AAA ATC GGG      1252
Tyr Asn Glu Lys Val Val Glu Val Arg Gly Leu Gly Ile Lys Ile Gly
                400                 405                 410

ATA GAT GTA TGG AAT GAA GGG ATT GAG ATC ACG GGC CCT GTA ATA GAA      1300
Ile Asp Val Trp Asn Glu Gly Ile Glu Ile Thr Gly Pro Val Ile Glu
            415                 420                 425

AGC GCC AAG ATT AGA GAA GCA ATT GAG AGA CTA ATG ATC AGT AAT GGT      1348
Ser Ala Lys Ile Arg Glu Ala Ile Glu Arg Leu Met Ile Ser Asn Gly
        430                 435                 440

TCT GAG GAA ATT ATA AAT ATT AGG GAT AGA GTA ATG GCT ATG AGC AAA      1396
Ser Glu Glu Ile Ile Asn Ile Arg Asp Arg Val Met Ala Met Ser Lys
    445                 450                 455

ATG GCT CAG AAT GCA ACA AAT GAA GGT GGA TCT TCG TGG AAC AAT CTC      1444
Met Ala Gln Asn Ala Thr Asn Glu Gly Gly Ser Ser Trp Asn Asn Leu
460                 465                 470                 475
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Thr Ala Leu Ile Gln His Ile Lys Asn Tyr Asn Leu Asn
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CAGAAATAAG TCCTTGCATT GTAACATGGT GTGTGTGTGT GTTTTTTTTC CACTTAATAA    1553

AATGAAGGAA TGGATGGATG GATGGATCTT AACTTTAAAA AAAAAAAAAA AAAA          1607
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(2) INFORMATION FOR SEQ ID NO:2:

(I) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 488 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Val Ala Thr Cys Asn Ser Gly Glu Ile Leu His Val Leu Phe Leu
 1               5                  10                  15

Pro Phe Leu Ser Ala Gly His Phe Ile Pro Leu Val Asn Ala Ala Arg
            20                  25                  30

Leu Phe Ala Ser Arg Gly Val Lys Ala Thr Ile Leu Thr Thr Pro His
        35                  40                  45

Asn Ala Leu Leu Phe Arg Ser Thr Ile Asp Asp Asp Val Arg Ile Ser
    50                  55                  60

Gly Phe Pro Ile Ser Ile Val Thr Ile Lys Phe Pro Ser Ala Glu Val
65                  70                  75                  80

Gly Leu Pro Glu Gly Ile Glu Ser Phe Asn Ser Ala Thr Ser Pro Glu
                85                  90                  95

Met Pro His Lys Ile Phe Tyr Ala Leu Ser Leu Leu Gln Lys Pro Met
            100                 105                 110

Glu Asp Lys Ile Arg Glu Leu Arg Pro Asp Cys Ile Phe Ser Asp Met
        115                 120                 125
```

Tyr Phe Pro Trp Thr Val Asp Ile Ala Asp Glu Leu His Ile Pro Arg
130 135 140

Ile Leu Tyr Asn Leu Ser Ala Tyr Met Cys Tyr Ser Ile Met His Asn
145 150 155 160

Leu Lys Val Tyr Arg Pro His Lys Gln Pro Asn Leu Asp Glu Ser Gln
165 170 175

Ser Phe Val Val Pro Gly Leu Pro Asp Glu Ile Lys Phe Lys Leu Ser
180 185 190

Gln Leu Thr Asp Asp Leu Arg Lys Ser Asp Asp Gln Lys Thr Val Phe
195 200 205

Asp Glu Leu Leu Glu Gln Val Glu Asp Ser Glu Glu Arg Ser Tyr Gly
210 215 220

Ile Val His Asp Thr Phe Tyr Glu Leu Glu Pro Ala Tyr Val Asp Tyr
225 230 235 240

Tyr Gln Lys Leu Lys Lys Pro Lys Cys Trp His Phe Gly Pro Leu Ser
245 250 255

His Phe Ala Ser Lys Ile Arg Ser Lys Glu Leu Ile Ser Glu His Asn
260 265 270

Asn Asn Glu Ile Val Ile Asp Trp Leu Asn Ala Gln Lys Pro Lys Ser
275 280 285

Val Leu Tyr Val Ser Phe Gly Ser Met Ala Arg Phe Pro Glu Ser Gln
290 295 300

Leu Asn Glu Ile Ala Gln Ala Leu Asp Ala Ser Asn Val Pro Phe Ile
305 310 315 320

Phe Val Leu Arg Pro Asn Glu Glu Thr Ala Ser Trp Leu Pro Val Gly
325 330 335

Asn Leu Glu Asp Lys Thr Lys Lys Gly Leu Tyr Ile Lys Gly Trp Val
340 345 350

Pro Gln Leu Thr Ile Met Glu His Ser Ala Thr Gly Gly Phe Met Thr
355 360 365

His Cys Gly Thr Asn Ser Val Leu Glu Ala Ile Thr Phe Gly Val Pro
370 375 380

Met Ile Thr Trp Pro Leu Tyr Ala Asp Gln Phe Tyr Asn Glu Lys Val
385 390 395 400

Val Glu Val Arg Gly Leu Gly Ile Lys Ile Gly Ile Asp Val Trp Asn
405 410 415

Glu Gly Ile Glu Ile Thr Gly Pro Val Ile Glu Ser Ala Lys Ile Arg
420 425 430

Glu Ala Ile Glu Arg Leu Met Ile Ser Asn Gly Ser Glu Glu Ile Ile
435 440 445

Asn Ile Arg Asp Arg Val Met Ala Met Ser Lys Met Ala Gln Asn Ala
450 455 460

Thr Asn Glu Gly Gly Ser Ser Trp Asn Asn Leu Thr Ala Leu Ile Gln
465 470 475 480

His Ile Lys Asn Tyr Asn Leu Asn
485

What is claimed is:

1. An isolated DNA sequence which encodes solanidine UDP-glucose glucosyltransferase (SGT).

2. The isolated DNA sequence of claim 1 selected from the group consisting of:

(a) an SGT DNA coding sequence identified as sequence ID No. 1; and

(b) a DNA sequence which hybridizes specifically to a SGT coding sequence of (a) or its complement under stringent conditions.

3. A recombinant DNA molecule containing an SGT DNA sequence or fragment thereof which is capable of being transcribed to form RNA, wherein said RNA is capable of inhibiting the production of SGT in a plant or plant cell in which said DNA molecule is transcribed, said plant or plant cell normally producing SGT, and wherein said DNA sequence or fragment thereof is operably linked to control sequences which effect its transcription into said RNA.

4. The recombinant DNA molecule of claim 3 wherein said DNA sequence or fragment thereof encodes SGT.

5. The recombinant DNA molecule of claim 3 wherein said DNA sequence or fragment thereof is in the 3' to 5' orientation.

6. A transgenic plant prepared by transforming plant cells with the recombinant DNA molecule of claim 3 and regenerating the plant from said transformed plant cells.

7. The transgenic plant of claim 6 which is potato or tomato.

8. A method of inhibiting SGT production in a plant cell which normally produces SGT, which comprises modifying said plant cell to contain an antisense nucleotide sequence complementary to an mRNA-encoding SGT.

9. A recombinant DNA molecule containing a sequence which encodes SGT.

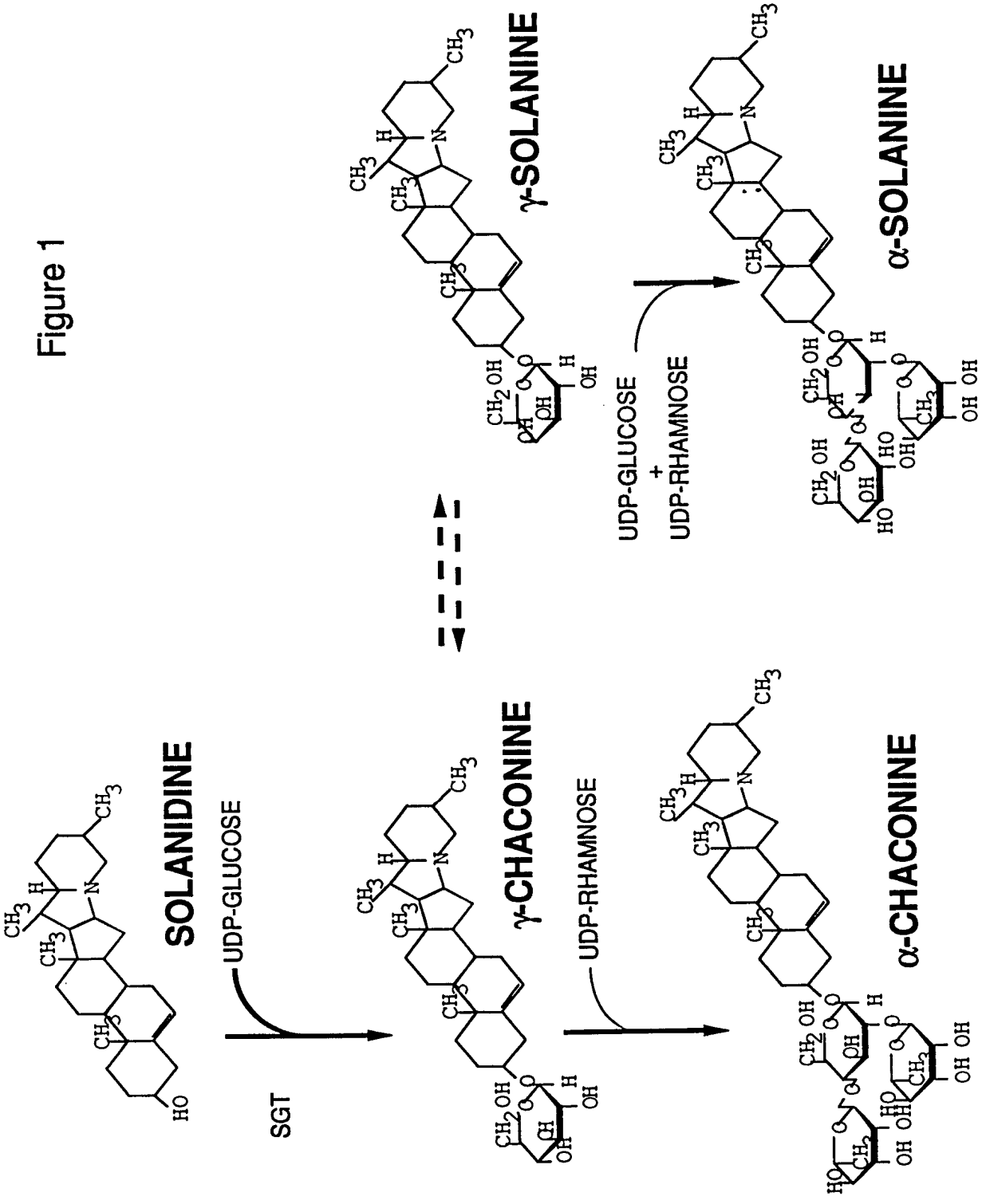
10. The recombinant DNA molecule of claim 9 wherein said molecule comprises an expression vector having a promoter and said DNA sequence is inserted in said vector downstream of said promoter and operatively associated therewith.

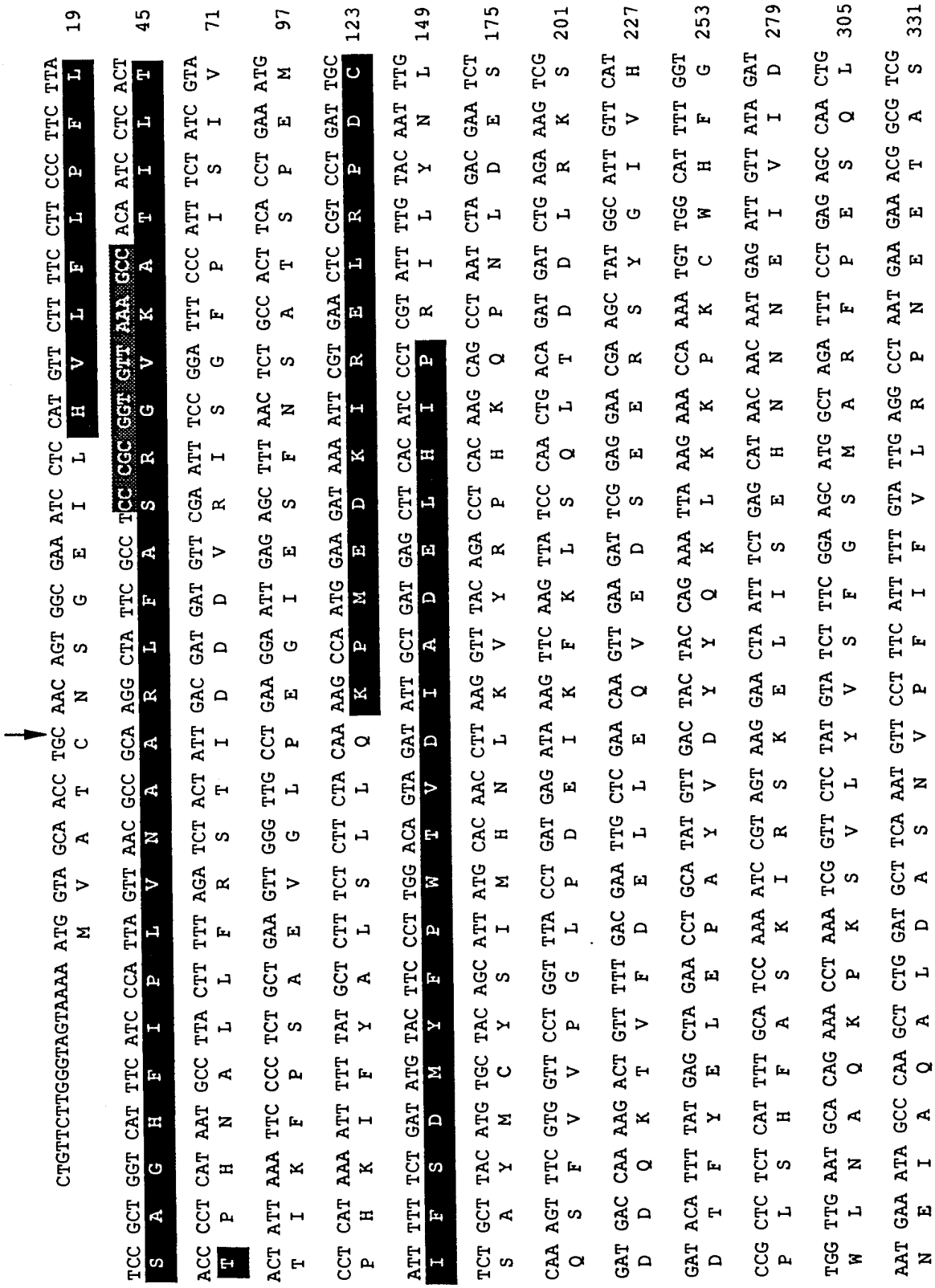
11. The recombinant DNA molecule of claim 10 wherein said promoter is a Granule Bound Starch Synthase (GBSS) promoter.

12. A cell transformed with the recombinant DNA molecule of claim 10.

13. A cell transformed with the recombinant DNA molecule of claim 11.

Figure 1


SOLANIDINE
UDP-GLUCOSE
SGT
γ-CHACONINE
UDP-RHAMNOSE
α-CHACONINE
γ-SOLANINE
UDP-GLUCOSE
+
UDP-RHAMNOSE
α-SOLANINE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TCC GCT GGT CAT TTC ATC CCA TTA GTT AAC GCC GCA AGG CTA TTC GCC TCC CGC GGT GTT AAA GCC ACA ATC CTC ACT
S A G H F I P L V N A A R L F A S R G V K A T I L T 45
ACC CCT CAT AAT GCC TTA CTT TTT AGA TCT ACT ATT GAC GAT GAT GTT CGA ATT TCC GGA TTT CCC ATT TCT ATC GTA
T P H N A L L F R S T I D D D V R I S G F P I S I V 71
ACT ATT AAA TTC CCC TCT GCT GAA GTT GGG TTG CCT GAA GGA ATT GAG AGC TTT AAC TCT GCC ACT TCA CCT GAA ATG
T I K F P S A E V G L P E G I E S F N S A T S P E M 97
CCT CAT AAA ATT TTT TAT GCT CTT TCT CTT CTA CAA AAG CCA ATG GAA GAT AAA ATT CGT GAA CTC CGT CCT GAT TGC
P H K I F Y A L S L L Q K P M E D K I R E L R P D C 123
ATT TTT TCT GAT ATG TAC TTC CCT TGG ACA GTA GAT ATT GCT GAT GAG CTT CAC ATC CCT CGT ATT TTG TAC AAT TTG
I F S D M Y F P W T V D I A D E L H I P R I L Y N L 149
TCT GCT TAC ATG TGC TAC AGC ATT ATG CAC AAC CTT AAG GTT TAC AGA CCT CAC AAG CAG CCT AAT CTA GAC GAA TCT
S A Y M C Y S I M H N L K V Y R P H K Q P N L D E S 175
CAA AGT TTC GTG GTT CCT GGT TTA CCT GAT GAG ATA AAG TTC AAG TTA TCC CAA CTG ACA GAT GAT CTG AGA AAG TCG
Q S F V V P G L P D E I K F K L S Q L T D D L R K S 201
GAT GAC CAA AAG ACT GTT TTT GAC GAA TTG CTC GAA CAA GTT GAA GAT TCG GAG GAA CGA AGC TAT GGC ATT GTT CAT
D D Q K T V F D E L L E Q V E D S E E R S Y G I V H 227
GAT ACA TTT TAT GAG CTA GAA CCT GCA TAT GTT GAC TAC TAC CAG AAA TTA AAG AAA CCA AAA TGT TGG CAT TTT GGT
D T F Y E L E P A Y Y V D Y Y Q K L K K P K C W H F G 253
CCG CTC TCT CAT TTT GCA TCC AAA ATC CGT AGT AAG GAA CTA ATT TCT GAG CAT AAC AAC AAT GAG ATT GTT ATA GAT
P L S H F A S K I R S K E L I S E H N N N E I V I D 279
TGG TTG AAT GCA CAG AAA CCT AAA TCG GTT CTC TAT GTA TCT TTC GGA AGC ATG GCT AGA TTT CCT GAG AGC CAA CTG
W L N A Q K P K S V L Y V S F G S M A R F P E S Q L 305
AAT GAA ATA GCC CAA GCT CTG GAT GCT TCA AAT GTT CCT TTC ATT TTT GTA TTG AGG CCT AAT GAA GAA ACG GCG TCG
N E I A Q A L D A S N V P F I F V L R P N E E T A S 331

Figure 2A

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TGG TTG CCA GTT GGT AAT TTA GAG GAC AAG ACT AAA AAG GGT TTG TAC ATC AAA GGG TGG GTC CCA CAG CTT ACG ATC 357
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CCA ATG ATA ACA TGG CCA CTT TAT GCT GAT CAA TTC TAC AAC GAG AAG GTA GTC GAG GTT AGG GGA TTG GGA ATC AAA 409
  P   M   I   T   W   P   L   Y   A   D   Q   F   Y   N   E   K   V   V   E   V   R   G   L   G   I   K

ATC GGG ATA GAT GTA TGG AAT GAA GGG ATT GAG ATC ACG GGC CCT GTA ATA GAA AGC GCC AAG ATT AGA GAA GCA ATT 435
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GAG AGA CTA ATG ATC AGT AAT GGT TCT GAG GAA ATT ATA AAT AGG GAT AGA GTA ATG GCT ATG AGC AAA ATG GCT 461
  E   R   L   M   I   S   N   G   S   E   E   I   I   N   I   R   D   R   V   M   A   M   S   K   M   A

CAG AAT GCA ACA AAT GAA GGT GGA TCT TCG TGG AAC AAT CTC ACT GCT CTC ATT CAA CAT ATC AAG AAT TAT AAT CTT 487
  Q   N   A   T   N   E   G   G   S   S   W   N   N   L   T   A   L   I   Q   H   I   K   N   Y   N   L

AAT TAG TTG AAG ACA GAA ATA AGT CCT TGC ATT GTA ACA TGG TGT GTG TGT GTG TTT TTT TTC CAC TTA ATA AAA TGA
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AGG AAT GGA TGG ATG GAT GGA TCT TAA CTT TAA AAA AAA AAA AAA AAA A
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Figure 2B

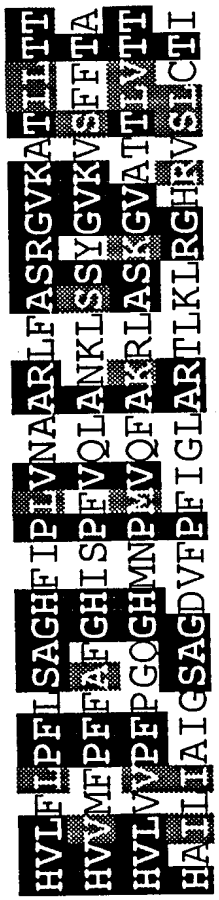

Figure 3

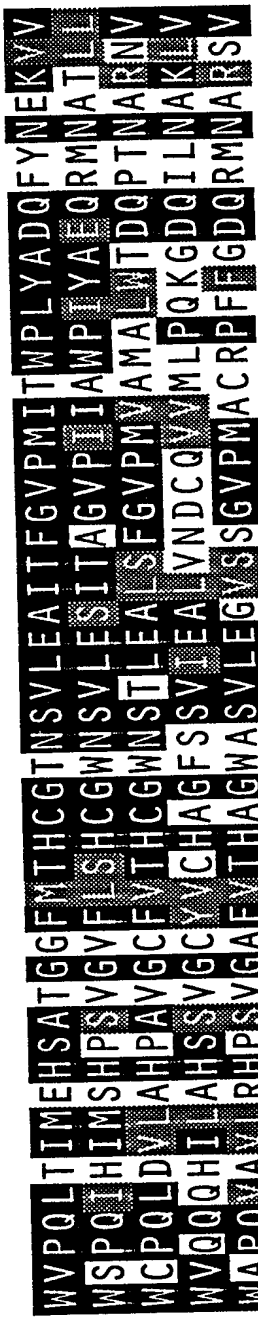

Figure 4

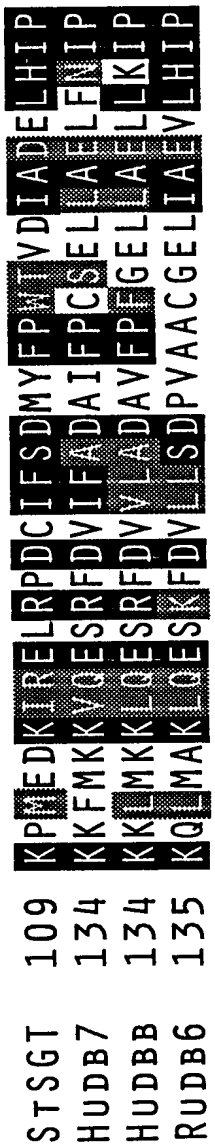
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Figure 5

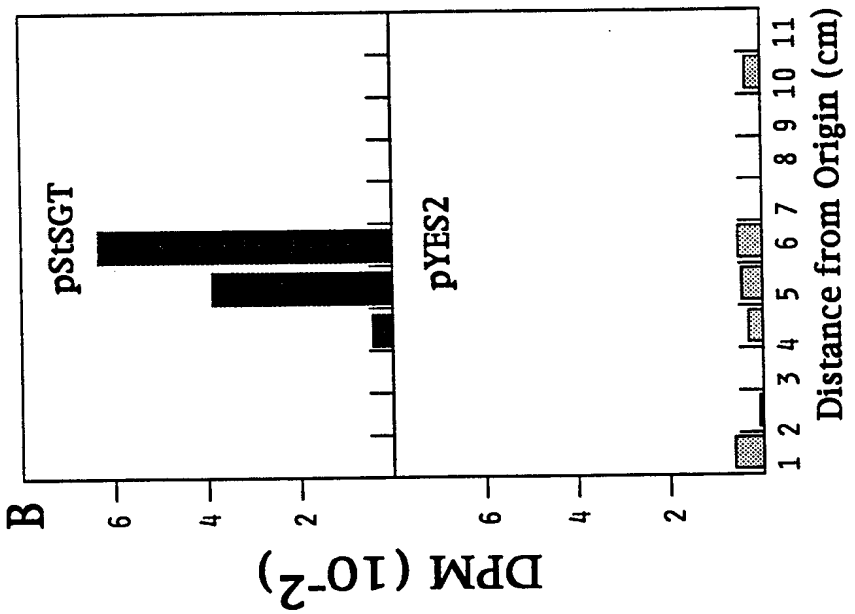


Figure 6B

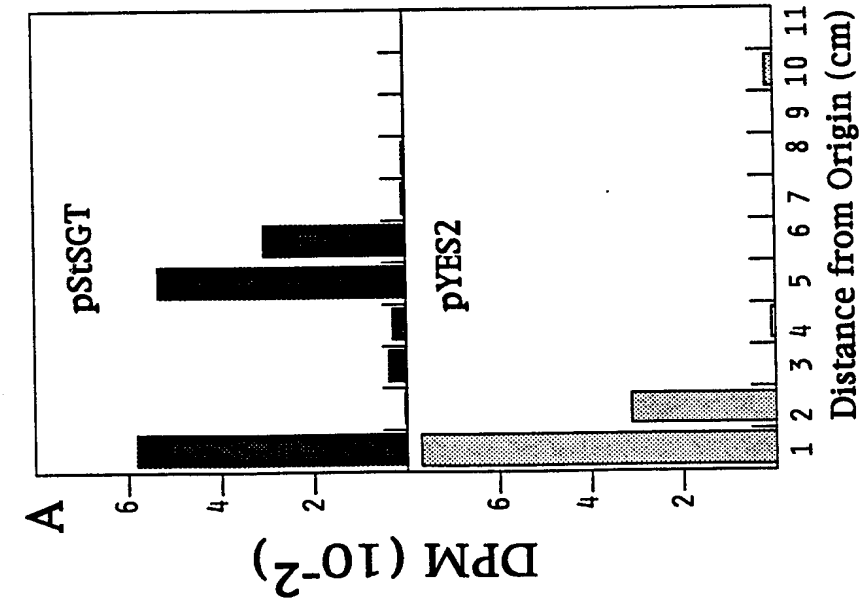
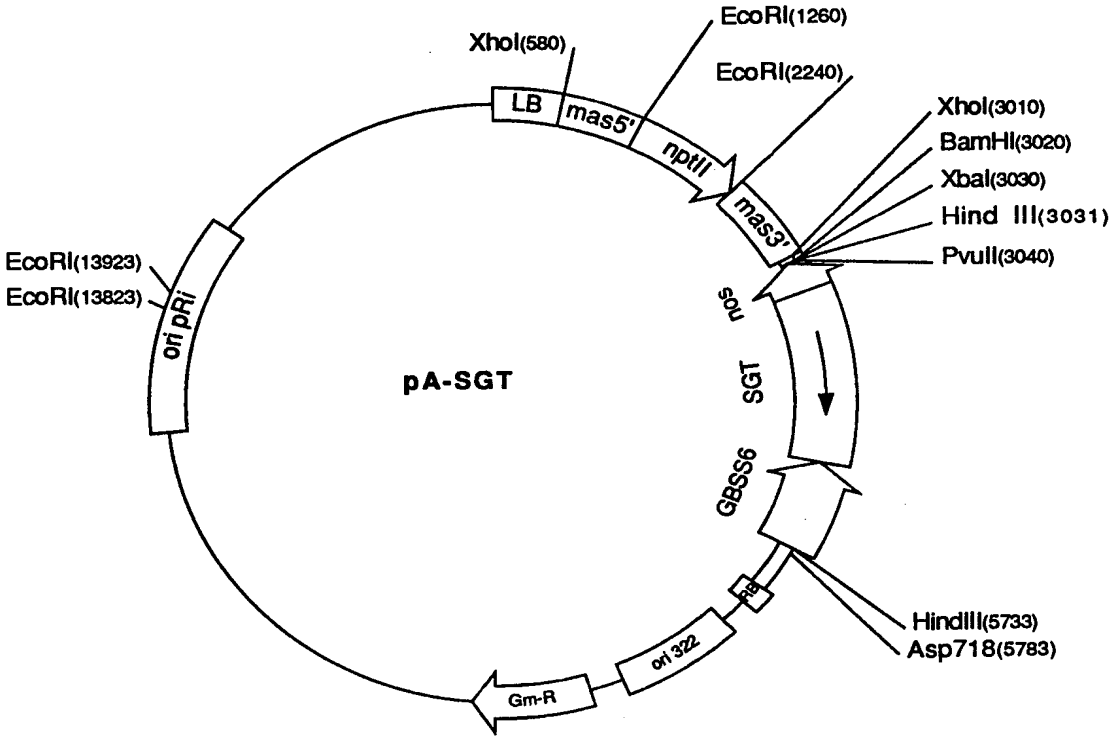


Figure 6A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EcoRI(1260)
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XhoI(3010)
BamHI(3020)
XbaI(3030)
Hind III(3031)
PvuII(3040)
LB
mas5'
nptII
mas3'
nos
SGT
GBSS6
HindIII(5733)
Asp718(5783)
ori322
Gm-R
ori pRi
EcoRI(13923)
EcoRI(13823)
pA-SGT

Figure 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01864

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A01H 5/00; C12N 5/14, 15/29, 15/54, 15/82
US CL :435/172.3, 320.1, 419; 536/23.2, 23.6; 800/205, DIG 42, DIG44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/172.3, 320.1, 419; 536/23.2, 23.6; 800/205, DIG 42, DIG44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN, BIOSIS, EMBASE, CA, WPIDS
search terms: solanidine, glucosyltransferase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	MOEHS et al. Cloning and expression of solanidine UDP-glucose glucosyltransferase from potato. The Plant Journal. February 1997, Vol. 11, No. 2, pages 227-236, see entire article.	1-4, 9-10 and 12 ---- 5-8, 11 and 13
A	BERGENSTRAHLE et al. Characterization of UDP-glucose:solanidine glucosyltransferase and UDP-galactose:solanidine galactosyltransferase from potato tuber. Plant Science. 1992, Vol. 84, pages 35-44, see entire article.	1-13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
06 APRIL 1998

Date of mailing of the international search report
02 JUN 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
AMY NELSON
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01864

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STAPLETON et al. Purification and characterization of solanidine glucosyltransferase from the potato (Solanum tuberosum). Journal of Agricultural and Food Chemistry. 1991, Vol. 39, pages 1187-1193, see entire article.	1-13
A	Database on BIOSIS, AN 97:309475. PACZKOWSKI et al. 'UDP-glucose:solasodine glucosyltransferase from eggplant (Solanum melongena L.) leaves: Partial purification and characterization'. Acta Biochimica Polonica. 1997, Vol. 44, No. 1, pages 43-54, see abstract.	1-13
A	Database on BIOSIS ,AN 92:325362. STAPLETON et al. 'Partial amino acid sequence of potato solanidine UDP-glucose glucosyltransferase purified by new anion-exchange and size exclusion media'. Protein Expression and Purification. 1992, Vol. 3, No. 2, pages 85-92, see abstract.	1-13